

Adenovirus-mediated Expression of Aquaporin-5 in Epithelial Cells*

(Received for publication, March 26, 1996, and in revised form, June 6, 1996)

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A recombinant adenovirus coding for rat aquaporin-5 was constructed and plaque purified. The recombinant adenovirus (AdrAQP5) mediated the expression of aquaporin-5 in rat and human salivary cell lines and in dog kidney cells *in vitro* as demonstrated by Northern blot and Western blot analyses, and by confocal microscopy after immunofluorescent labeling. In kidney cells, expression of the transgene was optimal if cells were infected at their basolateral surface, a phenomenon associated with the distribution of integrin receptors on these cells. The expressed aquaporin-5 protein was functionally active because viral-mediated gene transfer resulted in a significant increase in the osmotically directed net fluid secretion rate across monolayers of kidney cells. AdrAQP5 should provide an efficient and useful means to impart facilitated water permeability to cells lacking such a pathway.

Recently, a family of water channels has been identified that facilitate water permeability in a variety of tissues. These proteins have been named aquaporins (AQPs),¹ and five distinct members have been cloned from mammalian cells. AQP1 (previously named CHIP28) is a 28-kDa protein that is the prototype AQP and was first isolated from red blood cells (1–3). AQP5, recently cloned from rat submandibular glands, is present in the water transporting epithelia of lacrimal glands, trachea, eye, lung, and salivary glands (4). Topographically, it has been localized to the apical membranes in salivary and lacrimal glands (5).

Replication-deficient recombinant adenoviruses have been used for efficient gene transfer to mammalian cells *in vitro* and *in vivo* (6–12). We have previously demonstrated that such recombinant adenoviruses can effectively transfer genes to rat and human salivary cell lines *in vitro*, to all the major rat salivary glands *in vivo*, and to human minor glands *ex vivo* (13). The aim of the present study was to construct a recombinant adenovirus directing the expression of a functional AQP5 in order to examine the effects of introducing a facilitated water-permeable pathway into epithelial cells.

* This work was supported in part by National Institutes of Health Grants HL33991, HL48268, and EY11239. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¹ The abbreviations used are: AQP, aquaporin; MDCK, Madin-Darby canine kidney; PBS, phosphate-buffered saline; BSA, bovine serum albumin; CVL, crude viral lysate; m.o.i., multiplicity of infection; DB-cAMP, dibutyryl cyclic AMP.

EXPERIMENTAL PROCEDURES

Cell Culture—Human 293 cells were grown in Eagle's minimum essential medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM glutamine, 1,000 units/ml penicillin and 100 µg/ml streptomycin and routinely passaged every 2–3 days using citric saline (Biofluids, Rockville, MD). HSG cells (a gift of Dr. Mitsunabo Sato, Tokushima University, Japan), A5 cells (14), and SMIE cells (15) were grown in Eagle's minimum essential medium, McCoy's medium, and Dulbecco's modified Eagle's medium, respectively, supplemented as above and routinely passaged twice a week using trypsin (Biofluids, Rockville, MD). MDCK cells (a gift of Dr. Jared Grantham, University of Kansas, Kansas City, KS) were grown in Dulbecco's modified Eagle's medium/Ham's F-12 (1:1) medium containing 15 mM Hepes, pH 7.1–7.2, supplemented with 5% fetal bovine serum, 2 mM glutamine, 1,000 units/ml penicillin, and 100 µg/ml streptomycin and routinely passaged once a week using trypsin.

Direct Cloning of Rat AQP5 cDNA into pACCMV—The plasmid containing the rat aquaporin-5 cDNA (prAQP5) has been previously described (4). The adenovirus shuttle vector (pACCMV) and the plasmid containing the adenovirus serotype 5 genome (pJM17) were provided by Dr. C. Newgard (16) and Dr. F. Graham (17), respectively. The rAQP5 cDNA was inserted at the *EcoRI* and *BamHI* sites of pACCMV to yield the plasmid pCMVrAQP5 (Fig. 1).

Cotransfection of 293 Cells with pJM17 and pCMVrAQP5—293 cells (70–80% confluent) were cotransfected with 5 µg of pJM17 and 15 µg of pCMVrAQP5 using a calcium phosphate transfection system (Life Technologies, Inc.). After 5 h, the cells were washed three times with medium. Thereafter, the medium was replaced every 4–5 days. After 19 days the cells were harvested for cell lysate preparation. The cells were frozen and thawed three times and centrifuged at 5,000 × *g* for 10 min, and the crude viral lysate (CVL), containing the recombinant adenovirus (AdrAQP5), was used to infect confluent 293 cells. The virus was subsequently plaque purified and then purified by CsCl gradient centrifugation (16). The titer of the virus was determined by limiting dilution plaque assay.

Northern Blot Analysis—Poly(A) mRNA was purified from total RNA using an Oligotex[®] mRNA kit (Qiagen, Chatsworth, CA), electrophoresed on a 1.2% agarose gel containing 2.2 M formaldehyde, blotted onto nylon membranes (Schleicher & Schuell), and immobilized by cross-linking using UV light (Stratagene, La Jolla, CA). A 1-kilobase DNA fragment of the AQP5 cDNA was labeled with [³²P]dCTP and used as a probe. Prehybridization was performed at 68 °C for 30 min in QuickHyb solution (Stratagene) and hybridization at 68 °C for 3 h in QuickHyb solution containing 10 mg/ml salmon sperm DNA and the radiolabeled probe. The filters were washed at 60 °C with 0.1 × SSC/0.1% SDS. Blots were exposed to Kodak X-Omat AR film, with intensifying screens at –70 °C.

Western Blot Analysis—A peptide corresponding to the C-terminal 25 amino acids of AQP5 was synthesized, linked via an additional cysteine to a carrier protein, and used to immunize a rabbit (Lofstrand Labs Limited, Gaithersburg, MD). The antiserum was affinity purified using a sulfonate gel (Pierce) to which the peptide was attached. Crude plasma membranes were prepared from rat kidney, submandibular glands, and 293, A5, HSG, SMIE, and MDCK cells as described (18) except that a membrane pellet of 16,000 *g* was used and no β-mercaptoethanol was added to the sample buffer for SDS-polyacrylamide gel electrophoresis. The samples were analyzed by SDS-polyacrylamide gel electrophoresis using 12% acrylamide gels. Proteins were transferred to polyvinylidene difluoride membranes and immunolabeled using the affinity purified antibody to AQP5 at a 1:2000 dilution (see "Results"). The bound anti-

bodies were detected by using the ECL chemiluminescence method (Amersham Corp.). The resulting films were scanned using a laser densitometer, and the relative intensity of the monomeric AQP5 band peak was determined in $OD \times mm$.

Escherichia coli LacZ Gene Expression in MDCK Cells in Vitro—We utilized a recombinant adenovirus encoding β -galactosidase (Ad.RSV β Gal; Ref. 13) to evaluate the ability of MDCK cells to be infected by adenoviral vectors. The presence of β -galactosidase (the LacZ gene product) in Ad.RSV β Gal-infected MDCK cells was determined after 3 days using the X-Gal substrate as described (13).

Net Fluid Secretion Rate Measurement—The net fluid secretion rate in control MDCK cells and in MDCK cells infected at the basolateral side with either AdrAQP5 or Ad α 1AT (as an irrelevant recombinant adenovirus coding for α 1 antitrypsin; Ref. 13) was measured by two different methods. First, MDCK cells were grown to confluence on 24.5-mm diameter Transwell-Col culture chambers (Costar, Cambridge, MA) and washed on the apical side with 1 ml of oil, and then 1.5 ml of oil was added to the apical side (19). The medium of the basolateral chamber was replaced by 2.6 ml of fresh medium \pm 1 mM dibutyltryl (DBcAMP). After 24 h, the oil and any apically secreted water were collected and separated by centrifugation at 2,000 rpm for 5 min, and the secreted water was measured (19). The second method was a modification of this procedure. MDCK cells were grown as above. The apical fluid was replaced by 0.4 ml of hyperosmotic medium (400 mosm, *i.e.* 100 mM sucrose in medium), and the medium from the basolateral chamber was replaced by fresh isoosmotic medium (300 mosm). After 4 h, the liquid on the apical side was collected as above, its volume measured with a calibrated pipette, and the net fluid secretion rate was determined.

Localization of AQP5 Protein by Confocal Microscopy—MDCK cells, grown to confluence on Transwell-Col filters, were subjected to immunofluorescent staining for the AQP5 protein. Cells were rinsed with phosphate-buffered saline (PBS), pH 7.5, fixed with pre-chilled ($-70^\circ C$) 100% methanol on dry ice for 15 min, and blocked with 5% donkey serum (Jackson ImmunoResearch, West Grove, PA) in PBS containing 0.2% bovine serum albumin (BSA; Calbiochem, La Jolla, CA) for 20 min. Cells were incubated for 1 h with the affinity purified antibody directed against AQP5, diluted 1:50 in PBS/0.2% BSA, and then incubated for 20 min with the secondary antibody, fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Jackson ImmunoResearch), diluted 1:50 in PBS/0.2% BSA. After washing twice in PBS/0.2% BSA and twice in PBS alone, pieces of filter with attached cells were mounted on Silanated glass slides (Digene, Beltsville, MD) in Citofluor glycerol/PBS mixture (Ted Pella, Redding, CA) and examined in a Nikon Optiphot photomicroscope equipped with a Bio-Rad MRC-1000 laser scanning confocal imaging system (Bio-Rad, Cambridge, MA), using a Krypton/Argon laser as light source. The 488-nm light was used for excitation of the fluorescein isothiocyanate-labeled cells. Images were collected using a 60 \times oil immersion objective (PlanApo, Nikon, Japan). Cells were optically sectioned in *xy* and *xz* planes (parallel and perpendicular to the substratum).

Localization of Integrin Receptors by Confocal Microscopy—Immunofluorescent staining was performed as described above. Several anti-

bodies were used to examine the presence of integrin receptor subunits on MDCK cells: 1) rabbit anti-human integrin β -3 subunit polyclonal antibody (Chemicon Int. Inc., Temecula, CA), 2) mouse anti-human integrin α -V monoclonal antibody (Chemicon Int. Inc.), 3) monoclonal mouse antibody against human CD61 (= β -3; Immunotech Inc., Westbrook, ME), 4) anti-human integrin α -V, β -5 mouse monoclonal antibody (Life Technologies, Inc.), 5) rabbit anti-human integrin β -5 subunit polyclonal antibody (Chemicon Int. Inc.), 6) rabbit anti-human α -V, β -3/ α -V, β -5 (Life Technologies, Inc.). Only antibodies 2 and 3 gave immunopositive results on MDCK cells.

RESULTS

A replication-deficient recombinant adenovirus expressing AQP5, the mercurial-sensitive water channel reportedly localized to the apical membrane of acinar cells of rat submandib-

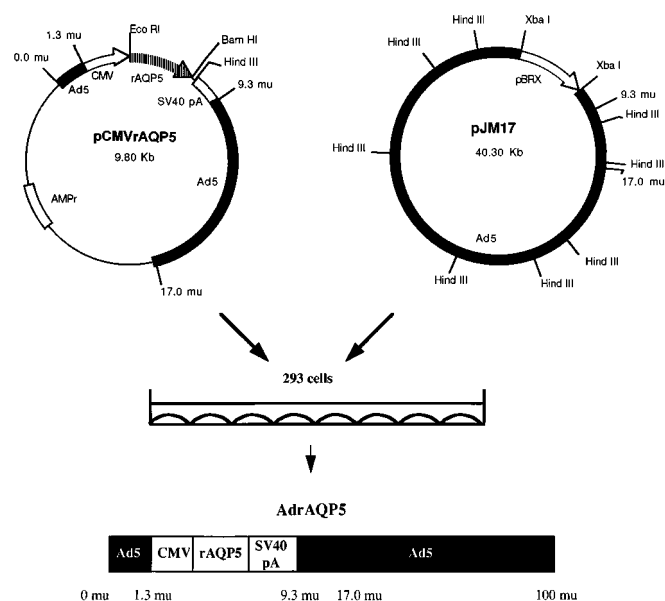


FIG. 1. Construction of a recombinant adenovirus containing the AQP5 cDNA. Rat AQP5 cDNA was cloned into the *Eco*RI and *Bam*HI sites of the multiple cloning site of the pACCMV vector. This vector includes some adenovirus sequence (Ad5), a CMV promoter, and a SV40 polyadenylation site (SV40 pA). pJM17 contains the adenovirus type 5 sequence. The homologous recombination between these two plasmids, after cotransfection into 293 cells, led to the formation of a replication-deficient recombinant adenovirus containing the AQP5 cDNA, AdrAQP5. *mu*, map units.

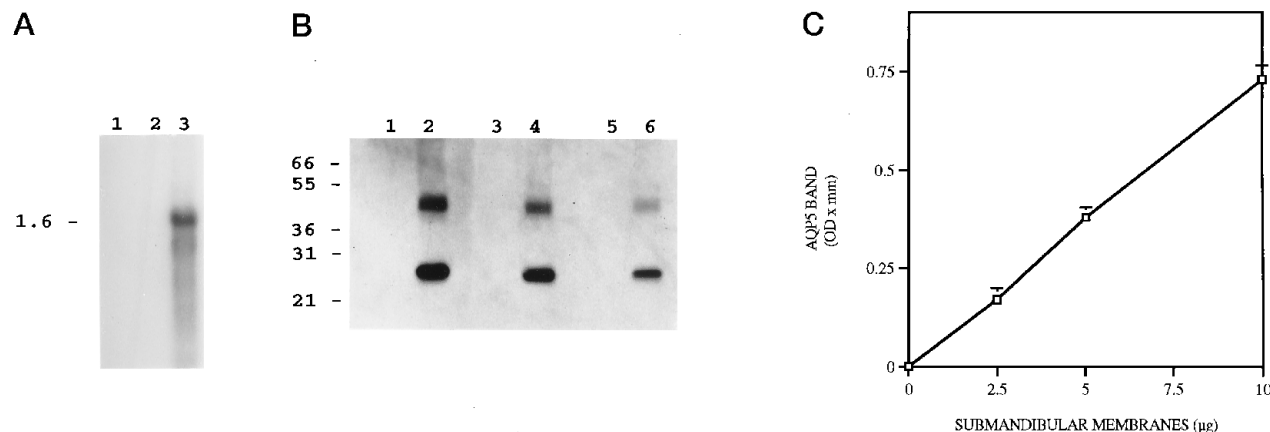


FIG. 2. Demonstration of AQP5 expression in AdrAQP5-infected 293 cells. A, Northern blot analysis was performed using 1 μ g of poly(A) mRNA from uninfected 293 cells (lane 1), 293 cells infected with the irrelevant virus Ad α 1AT (lane 2), or AdrAQP5 (lane 3) (see "Experimental Procedures" for details). B, Western blots were performed using 10 μ g of crude rat kidney membranes (lanes 1, 3, and 5) or 10 μ g of crude rat submandibular gland membranes (lanes 2, 4, and 6), and the affinity purified antibody was used at dilutions 1:500 (lanes 1 and 2), 1:1000 (lanes 3 and 4), and 1:2000 (lanes 5 and 6). C, Western blot analysis was performed using 2.5, 5.0, and 10 μ g of crude rat submandibular membranes and the affinity purified antibody diluted at 1:2000. The film of the Western blot was scanned as described under "Experimental Procedures." The results are the means \pm S.E. of three separate determinations.

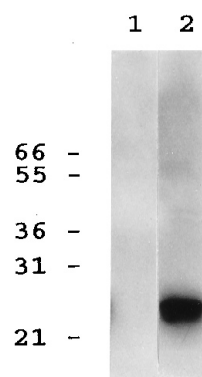


FIG. 3. **Expression of AQP5 protein in 293 cells infected with AdrAQP5.** 10 μ g of crude membranes from uninfected 293 cells (lane 1) or 293 cells infected with AdrAQP5 (lane 2) were analyzed by Western blot.

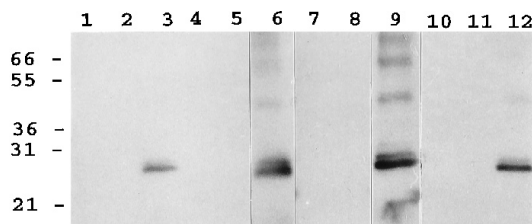


FIG. 4. **AQP5 expression in AdrAQP5-infected MDCK, HSG, A5, and SMIE cells.** 10 μ g of crude membranes from MDCK (lanes 1–3), HSG (lanes 4–6), A5 (lanes 7–9), and SMIE (lanes 10–12) cells were analyzed by Western blot. The cells were either uninfected (lanes 1, 4, 7, and 10), infected at a m.o.i. of 10 of Ad α 1AT (lanes 2, 5, 8, and 11), or infected at a m.o.i. of 10 of AdrAQP5 (lanes 3, 6, 9, and 12).

ular glands (5), was constructed by homologous recombination between the pCMVrAQP5 and pJM17 plasmids in 293 cells (see "Experimental Procedures" and Fig. 1). Two weeks following the cotransfection, there was evidence of cytopathic effects in the 293 cells. At 19 days, the cells were harvested, and CVL, containing the recombinant adenovirus AdrAQP5, was used to infect other 293 cells.

Twenty-four hours after subsequent infection of confluent 293 cells, poly(A) mRNA was obtained from total RNA, and Northern blot analysis was performed (Fig. 2A). No AQP5 transcript was detected in untreated control 293 cells or in 293 cells infected with Ad α 1AT (*i.e.* the irrelevant virus coding for alpha1-antitrypsin). However, in 293 cells infected with the CVL, abundant amounts of a hybridization-positive transcript of \sim 1.6 kilobases were detected.

To detect the production of AQP5 protein, we developed an antibody directed against a peptide corresponding to the C-terminal 25 amino acids of AQP5. This antibody was affinity purified, and its specificity was determined by Western blot analysis. Different dilutions were used to detect the native AQP5 found in rat submandibular gland membranes (Fig. 2B). Rat submandibular gland but not rat kidney membranes showed two regions of immunoreactivity; one band \sim of 27 kDa the other band of \sim 45 kDa. The former corresponds to a non-glycosylated AQP5 monomer. The latter does not represent a glycosylated form of AQP5, because it did not disappear after *N*-glycanase treatment, whereas the higher molecular weight form of AQP1 (1) was converted to a \sim 28-kDa band after such treatment (data not shown). When increasing concentrations of submandibular membranes were electrophoresed, the upper band increased in a linear fashion but with a slope markedly different from the monomeric form (data not shown). Thus, the upper band likely represents a dimer of AQP5 that runs anomalously on SDS-polyacrylamide gel electrophoresis; a known

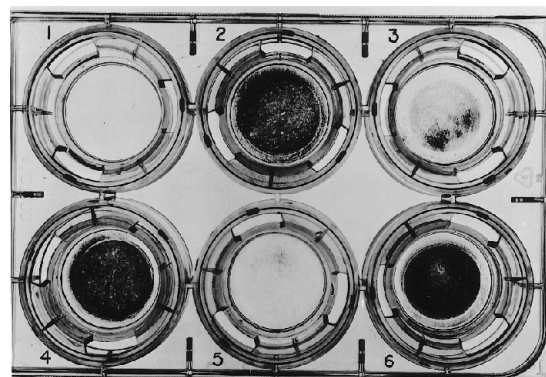


FIG. 5. **Efficiency of adenovirus infection in MDCK cells using Ad.RSV β Gal.** MDCK cells grown on filters were not infected (well 1) or infected at the basolateral (well 2) or apical (well 3) side or both sides (well 4) at a m.o.i. of 100 of Ad.RSV β Gal or infected at the apical (well 5) or basolateral (well 6) side at a m.o.i. of 10 of Ad.RSV β Gal. β -Galactosidase activity was visualized as described under "Experimental Procedures."

characteristic of integral membrane proteins (20). Furthermore, Denker *et al.* (1) showed that after SDS-polyacrylamide gel electrophoresis, AQP1 also was detected as both a monomeric band (\sim 28 kDa) plus several oligomers. Similar Western blot patterns were seen with 1:500, 1:1000, and 1:2000 dilutions of the affinity-purified antibody. Based on these results the affinity-purified antibody was routinely used at the dilution 1:2000. As shown in Fig. 2C, under these conditions the detection of native AQP5 in rat submandibular gland membranes was linear between 2.5–10 μ g of membrane protein.

We next used this affinity purified antibody to monitor the plaque purification of AdrAQP5. When 293 cells were infected with CVL, AQP5 protein could be readily detected in crude membranes (not shown). All plaques selected gave positive results showing expression of AQP5. Subsequently, we chose one plaque for large scale production and purification on CsCl gradients. The titer of the final purified AdrAQP5, as determined by plaque assay, was 8.5×10^{11} plaque-forming units/ml. As shown in Fig. 3; when 293 cells were infected with AdrAQP5, AQP5 protein could be detected in crude membrane preparations after Western blot analysis.

We next infected several epithelial cell lines with AdrAQP5. MDCK is a well studied dog kidney-derived cell line, whereas HSG, A5, and SMIE cells are derived from salivary epithelia (human, rat, and rat, respectively). Forty-eight hours after infection, cells were harvested and crude membranes were prepared. Western blot analysis revealed the presence of AQP5 protein in all AdrAQP5-infected epithelial cell lines (Fig. 4). Conversely, no AQP5 protein band could be detected in uninfected cells or in cells that were infected with the irrelevant virus, Ad α 1AT.

MDCK cells grown on collagen-coated permeable filters form polarized monolayers with distinct apical and basolateral membranes (21). MDCK cells were either not infected or infected for 3 days at a multiplicity of infection (m.o.i.) of either 10 or 100 of Ad.RSV β Gal at either the apical or basolateral side or both sides. No β -galactosidase activity was detected in non-infected MDCK cells (Fig. 5, well 1). When MDCK cells were infected at the apical side at a m.o.i. of either 100 (Fig. 5, well 3) or 10 (Fig. 5, well 5) of Ad.RSV β Gal, little β -galactosidase activity was apparent. However, when MDCK cells were infected at the basolateral side at a m.o.i. of either 100 (Fig. 5, well 2) or 10 (Fig. 5, well 6) of Ad.RSV β Gal or at both apical and basolateral sides at a m.o.i. of 100 of Ad.RSV β Gal (Fig. 5, well 4), considerable β -galactosidase activity was seen.

Because integrin receptors are known to mediate the entry of

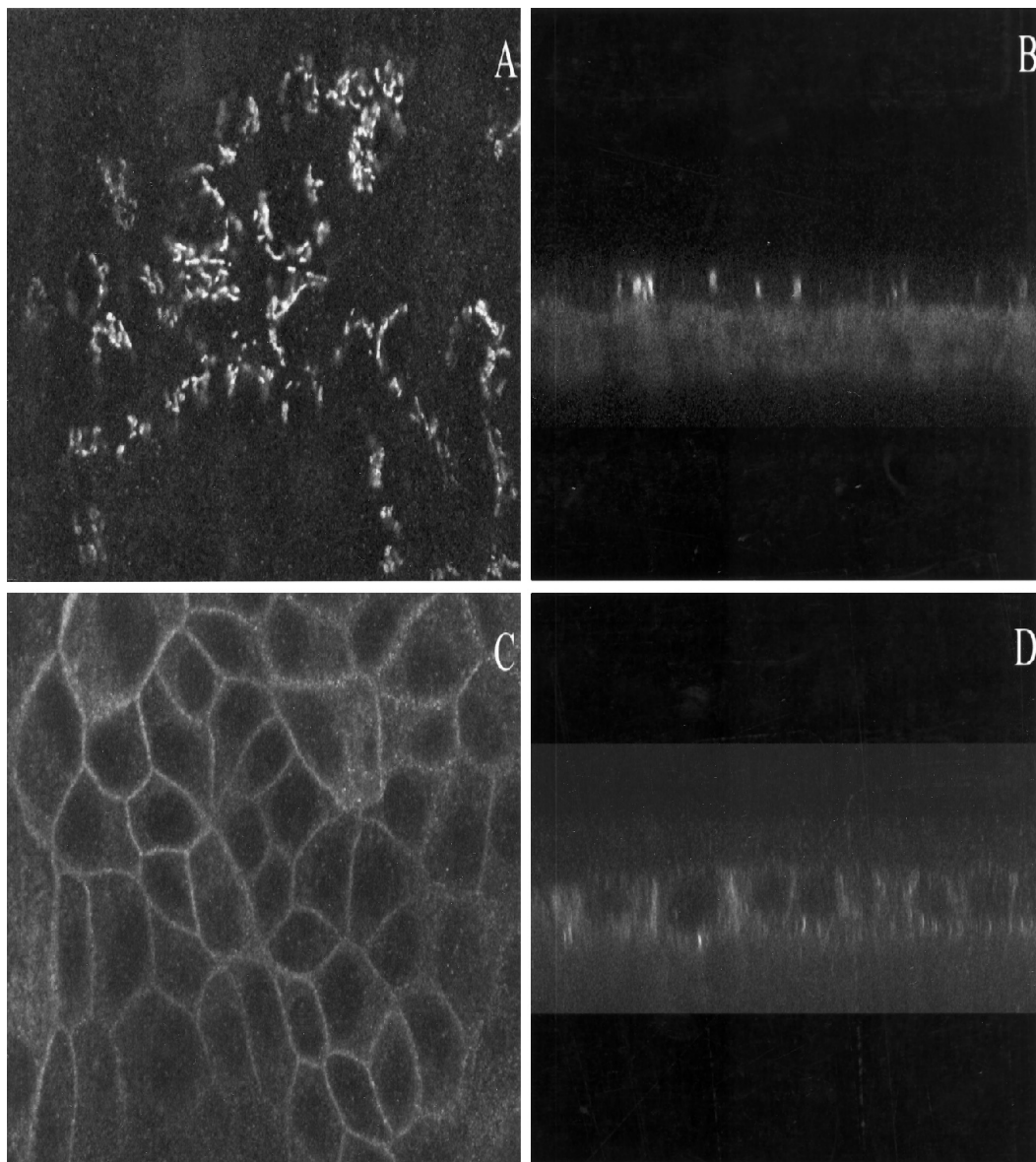


FIG. 6. **Localization of integrin receptors in MDCK cells.** MDCK cells grown on filters were labeled using mouse anti-human integrin alpha-V monoclonal antibody (*A* and *B*) and mouse anti-human beta-3 monoclonal antibody (*C* and *D*) integrin protein and examined by confocal microscopy as described under "Experimental Procedures." Micrographs of horizontal (*xy*; *A* and *C*), and vertical (*xz*; *B* and *D*) optical sections through cells are shown.

adenoviruses into epithelial cells (22), we speculated that the above differences in β -galactosidase expression were due to the distribution of integrin receptors in these cells. We looked for the presence of such receptors in polarized MDCK cells by confocal microscopy using antibodies directed against alpha-V, beta-3, and beta-5 integrin subunit proteins. Using the antibodies available, we observed that MDCK cells expressed the alpha-V, beta-3 integrin protein subunits on the plasma membranes (Fig. 6, *A* and *C*) and more specifically at their lateral domain, as shown by vertical optical sections of the cells (Fig. 6, *B* and *D*). No evidence for the beta-5 integrin subunit was found.

We next examined cells grown in this manner to localize the cellular sites of AQP5 expression by confocal microscopy. Uninfected cells and cells infected at the basolateral side at a m.o.i. of 100 of Ad α 1AT showed no AQP5 expression (Fig. 7, *A–D*). However, when cells were infected at a m.o.i. of 100 of AdrAQP5, AQP5 protein was highly expressed. AQP5 expression in these cells was detected at both apical and basolateral sides of the plasma membrane (Fig. 7, *E* and *F*).

In order to determine if the AQP5 expressed in AdrAQP5-infected cells affected their water permeability, we measured the transepithelial net fluid secretion rate in MDCK cells. MDCK cells, when grown on collagen-coated permeable filters, form a tight epithelium with a moderately high transepithelial resistance (21). It has been previously shown (19) that a low level of water secretion across this *in vitro* epithelial monolayer can be measured when the apical medium is replaced with mineral oil. Further, DBcAMP increases this net fluid secretion rate significantly (19). We used this method to examine *in situ* the function of AQP5. As shown in Table I, the measured rates were low but quite similar to those described by Neufeld *et al.* (Ref. 19; $\sim 0.3 \mu\text{l}/\text{cm}^2 \cdot \text{h}$). However, no differences were seen in the net fluid secretion rates between control MDCK cells and those infected with either Ad α 1AT or AdrAQP5. We modified this experimental system to measure the net fluid secretion rate of MDCK cells under more physiological conditions, *i.e.* in the presence of a transepithelial (basolateral to apical) osmotic gradient. After 4 h of incubation, the net fluid secretion rates of control and Ad α 1AT-infected MDCK cells were not signifi-

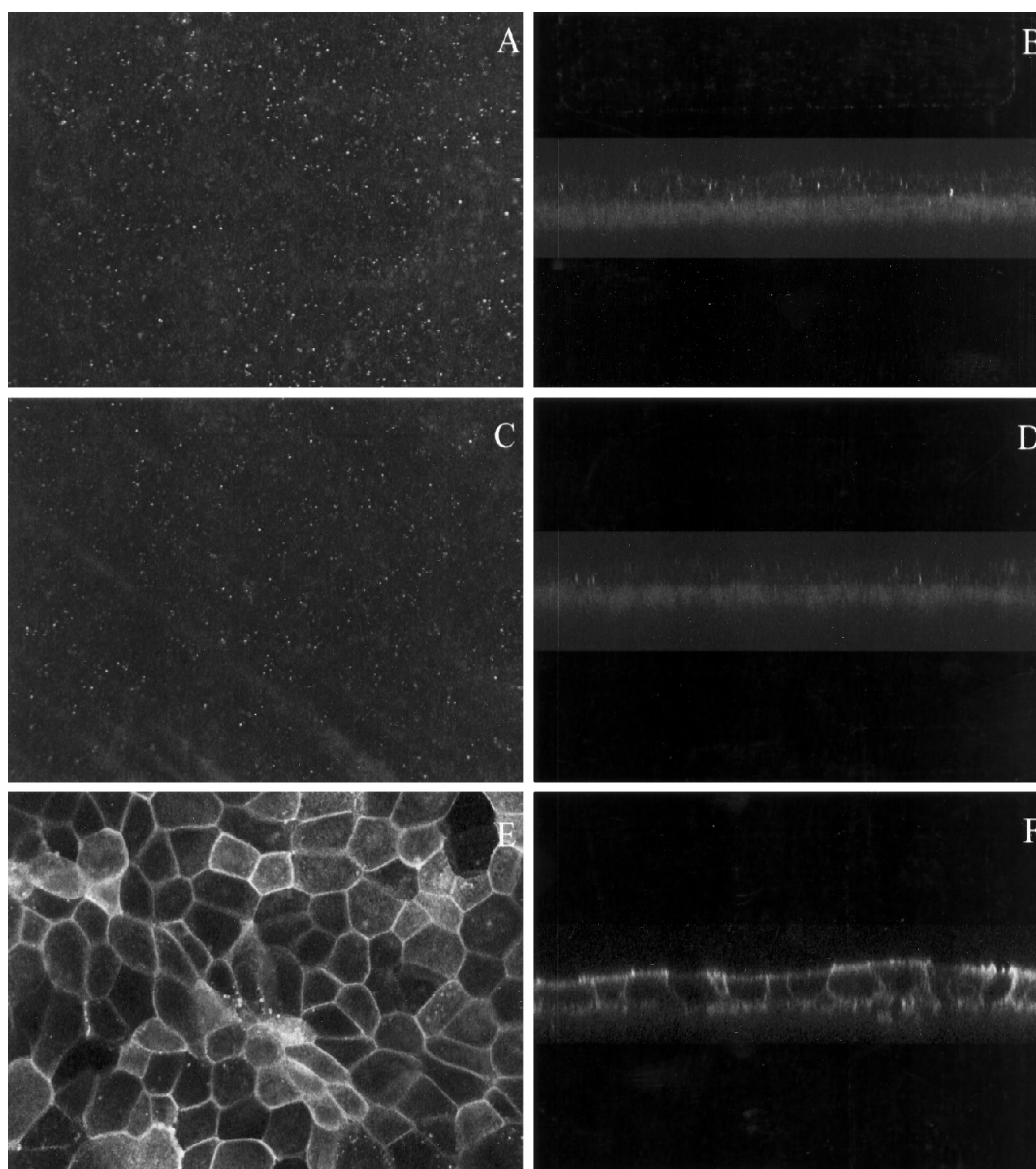


FIG. 7. **Confocal microscopic localization of AQP5 expression in polarized MDCK cells.** Confluent MDCK cells, grown on filters, were not infected (*A* and *B*) or infected at the basolateral side at a m.o.i. of 100 of Ad α 1AT (*C* and *D*) or at a m.o.i. of 100 of AdrAQP5 (*E* and *F*). The cells were then labeled for AQP5 and examined by confocal microscopy as described under "Experimental Procedures." Horizontal (*xy*; *A*, *C*, and *E*) and vertical (*xz*; *B*, *D*, and *F*) images are shown.

TABLE I
Net fluid secretion rates across MDCK monolayers

Net fluid secretion rates of control, Ad α 1AT- (m.o.i. of 100) and AdrAQP5- (m.o.i. of 100) infected MDCK cells were measured using either mineral oil at the apical side and medium without or with 1 mM DBcAMP at the basolateral side or using a hyperosmotic medium (400 mosm) at the apical side and isoosmotic medium at the basolateral side. The results are expressed as fluid flow in μ l secreted/cm²/hour and are the means \pm S.E. of three separate experiments performed in triplicate.

	Water flow		
	Control (oil)	DBcAMP (oil)	400 mosm
		μ l/cm ² · h	
Uninfected	0.31 \pm 0.03	0.54 \pm 0.06	1.75 \pm 0.31
+ Ad α 1AT	0.23 \pm 0.04	0.49 \pm 0.03	1.91 \pm 0.21
+ AdrAQP5	0.30 \pm 0.02	0.45 \pm 0.02	7.11 \pm 0.89

cantly different from each other but were considerably higher (\sim 5–6 \times) than those described by Neufeld *et al.* (19). Importantly for the present studies, the net fluid secretion rate across AdrAQP5-infected MDCK cells was increased an additional

4-fold (Table I). These results demonstrated that the cells infected with AdrAQP5 show considerably more water permeability in response to an osmotic challenge than control cells or cells infected with an irrelevant virus.

DISCUSSION

We have successfully constructed a replication-deficient recombinant adenovirus expressing AQP5. When 293 cells were infected with CVL, Northern blot analysis revealed a hybridization-positive band of \sim 1.6 kilobases. The \sim 1.6-kilobase transcript is of comparable size to the AQP5 transcript originally found in rat salivary glands, lacrimal gland, trachea, eye, and lung (4).

We developed (and affinity-purified) an antibody directed against a peptide corresponding to the C-terminal 25 amino acids of AQP5. In uninfected 293 cells, AQP5 protein was not detected, whereas in 293 cells infected with AdrAQP5 CVL, AQP5 protein was readily detected. We used purified AdrAQP5 to infect other cell lines *in vitro*. In infected MDCK, HSG, A5, and SMIE cells, the primary immunopositive band observed in

Western blots was the monomeric nonglycosylated AQP5. In salivary epithelial cells (HSG, A5, and SMIE) additional fainter immunopositive bands of higher molecular weight than monomeric AQP5 were detected. These might reflect the presence of aggregates between AQP5 protein or between AQP5 and other proteins.

Using a recombinant adenovirus containing the LacZ (Ad.RSV β Gal) gene encoding β -galactosidase, we showed that the infection of MDCK cells grown on permeable membranes was much more efficient when the cells were infected at their basolateral side. Because integrin receptors mediate the entry of adenoviruses in cells (22), we examined their localization in MDCK cells. Immunofluorescent labeling of relevant integrin receptors showed that the α -V, β -3 integrin subunits were localized at the lateral side of polarized MDCK cells, consistent with the higher levels of β -galactosidase activity seen when MDCK cells were infected with Ad.RSV β Gal at their basolateral side.

Because AQP5 appears naturally localized to the apical membranes of expressing cells, we examined the sites of expression of virus-directed AQP5 in MDCK cells grown as a polarized monolayer on filters. In uninfected cells and cells infected with an irrelevant virus there was no AQP5 expression observed. When the cells were infected with AdrAQP5 at a m.o.i. of 100, AQP5 labeling was detected at the apical membrane as well as at the basolateral membrane. These results may reflect an inability of the MDCK cells to sort AQP5 to the "correct membrane" *in vitro* (as has been seen with AQP2 sorting in LLC-PK1 cells *in vitro*; Ref. 23). Alternatively, the inserted AQP5 cDNA may lack a necessary routing signal.

The function of the recombinant AQP5 protein was determined *in vitro* in polarized MDCK cells (which are devoid of any endogenous AQP5) that were infected with AdrAQP5. As shown by Neufeld *et al.*, (19) when basal transepithelial fluid secretion is measured in MDCK cells, low levels of fluid movement could be detected and can be increased ~2-fold by DBcAMP. We observed similar results in uninfected MDCK cells and MDCK cells infected with either the irrelevant virus or AdrAQP5. Thus the presence of AQP5 did not modify fluid secretion under these experimental conditions. However, when net fluid secretion was evaluated under more physiological conditions, *i.e.* in the presence of a transepithelial osmotic gradient, the net fluid secretion rates of uninfected MDCK cells and of MDCK cells infected with the irrelevant virus were increased 5–6-fold as compared with the rates observed above. Importantly, in the MDCK cells infected with AdrAQP5 and exposed to this osmotic gradient there was an additional 4-fold higher net fluid secretion rate. These data clearly suggest that the recombinant AQP5 was indeed functional.

In conclusion, the construction of a replication-deficient recombinant adenovirus encoding AQP5 was successfully achieved. The recombinant virus can mediate the transfer and subsequent expression of the AQP5 cDNA *in vitro* as shown by Northern blot analysis, Western blot analysis, and confocal microscopy. Moreover, the function of the recombinant AQP5 was demonstrated by measurement of net fluid secretion rates in a cultured relatively "tight" epithelial cell line. To our knowledge, these data represent the first reported expression of functional AQP5 protein in mammalian epithelial cells *in vitro*. Thus, this virus should provide an efficient and useful means to impart facilitated water permeability to cells lacking such a pathway.

REFERENCES

- Denker, B. M., Smith, B. L., Kuhajda, F. P., and Agre, P. (1988) *J. Biol. Chem.* **263**, 15634–15642
- Preston, G. M., and Agre, P. (1991) *Proc. Natl. Acad. Sci. U. S. A.* **88**, 11110–11114
- Preston, G. M., Carroll, T. P., Guggino, W. B., and Agre, P. (1992) *Science* **256**, 385–387
- Raina, S., Preston, G. M., Guggino, W. B., and Agre, P. (1995) *J. Biol. Chem.* **270**, 1908–1912
- Agre, P., Brown, D., and Nielsen, S. (1995) *Curr. Opin. Cell Biol.* **7**, 472–483
- Rosenfeld, M. A., Siegfried, W., Yoshimura, K., Yoneyama, K., Fukuyama, M., Stier, L. E., Paako, P. K., Gilardi, P., Stratford-Perricaudet, L. D., Perricaudet, M., Jallat, S., Pavirani, A., Lecocq, J.-P., and Crystal, R. G. (1991) *Science* **252**, 431–434
- Gomez-Foix, A. M., Coats, W. S., Baque, S., Alam, T., Gerard, R. D., and Newgard, C. B. (1992) *J. Biol. Chem.* **267**, 25129–25134
- Mitani, K., Graham, F. L., and Caskey, T. (1994) *Human Gene Ther.* **5**, 941–948
- Kozarsky, K. F., McKinley, D. R., Austin, L. L., Raper, S. E., Stratford-Perricaudet, L. D., and Wilson, J. M. (1994) *J. Biol. Chem.* **269**, 13695–13702
- Lee, S. W., Trapnell, B. C., Rade, J. J., Virmani, R., and Dichek, D. A. (1993) *Circ. Res.* **73**, 797–807
- Crystal, R. G., McElvaney, N. G., Rosenfeld, M. A., Chu, C.-S., Mastrangeli, A., Hay, J. G., Brody, S. L., Jaffe, H. A., Eissa, N. T., and Danel, C. (1994) *Nature Gen.* **8**, 42–51
- Bajocchi, G., Feldman, S., Crystal, R. G., and Mastrangeli, A. (1993) *Nat. Genet.* **3**, 224–229
- Mastrangeli, A., O'Connell, B., Alabib, W., Fox, P. C., Baum, B. J., and Crystal, R. G. (1994) *Am. J. Physiol.* **266**, G1146–G1155
- He, X., Ship, J., Wu, X., Brown, A. M., and Wellner, R. B. (1989) *J. Cell. Physiol.* **138**, 5294–5299
- He, X. J., Frank, D. P., and Tabak, L. A. (1990) *Biochem. Biophys. Res. Commun.* **170**, 336–343
- Becker, T. C., Noel, R. J., Coats, W. S., Gomez-Foix, A. M., Alam, T., Gerard, R. D., and Newgard, C. B. (1994) *Methods Cell Biol.* **43**, 161–189
- McGrory, W. J., Bautista, D. S., and Graham, F. L. (1988) *Virology* **163**, 614–617
- Dai, Y., Ambudkar, I. S., Horn, V. J., Yeh, C.-H., Kousvelari, E. E., Wall, S. J., Li, M., Yasuda, R. P., Wolfe, B. B., and Baum, B. J. (1991) *Am. J. Physiol.* **261**, C1063–C1073
- Neufeld, T. K., Grant, M. E., and Grantham, J. J. (1991) *J. Tissue Cult. Methods* **13**, 229–234
- Silverberg, M., and Marchesi, V. T. (1978) *J. Biol. Chem.* **253**, 95–98
- Cereijido, M., Robbins, E. S., Dolan, W. J., Rotunno, C. A., and Sabatini, D. D. (1978) *J. Cell Biol.* **77**, 853–880
- Goldman, M. J., and Wilson, J. M. (1995) *J. Virol.* **69**, 5951–5958
- Katsura, T., Verbavatz, J.-M., Farinas, J., Ma, T., Ausiello, D. A., Verkman, A. S., and Brown, D. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 7212–7216